

Sentinel Chicken Seroconversions Track Tangential Transmission of West Nile Virus to Humans in the Greater Los Angeles Area of California

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Abstract. In Los Angeles, California, West Nile virus (WNV) has followed a pattern of emergence, amplification, subsidence, and resurgence. A time series cross-correlation analysis of human case counts and sentinel chicken seroconversions revealed temporal concordance indicating that chicken seroconversions tracked tangential transmission of WNV from the basic passeriform-*Culex* amplification cycle to humans rather than antecedent enzootic amplification. Sentinel seroconversions provided the location and time of transmission as opposed to human cases, which frequently were reported late and were assumed to be acquired 2–14 days before disease onset at their residence. Cox models revealed that warming degree-days were associated with the increased risk of seroconversion, whereas elevated herd immunity in peridomestic birds dampened seroconversion risk. Spatially, surveillance data collected within a 5 km radius of flock locations 15–28 days before the bleed date were most predictive of a seroconversion. In urban Los Angeles, sentinel chicken seroconversions could be used as an outcome measure in decision support for emergency intervention.

INTRODUCTION

Sentinel chickens have been a part of the California mosquito-borne encephalitis virus monitoring program since its inception, but historically were limited to three flocks of 10 birds each in Los Angeles, thereby limiting effectiveness. Spatial coverage was extended in 2003 to track the invasion of West Nile virus (*Flaviviridae*, *Flavivirus*, WNV). Sentinel chickens have the advantages of monitoring enzootic transmission events at a specific location and are more cost-effective than other arboviral surveillance indicators.¹ Chickens produce WNV-specific antibody as early as 5 days post-infection, and enzyme immunoassays (EIA) can detect seroconversions as early as 7–10 days post-infection.² Therefore, when bled at frequent intervals chickens delineate not only the place but also the approximate time of infection. An additional advantage of using sentinel chickens is that their low viremia is unlikely to infect *Culex* mosquito vectors,^{3–5} thereby reducing the ethical dilemma of placing a competent host near human residences.

Defining the spatial dimensions of amplification and tangential transmission of WNV over urban landscapes has been problematic, but necessary for mosquito control. Initial analysis of WNV dispersion in Los Angeles indicated spatial congruency among clusters of WNV-positive dead birds, elevated *Culex pipiens quinquefasciatus* Say infection, and the incidence of human cases.⁶ However, human case data were difficult to use, because: 1) the protection of patient privacy resulted in generalizing the location of infection to zip code, and 2) the mobility of humans made it difficult to determine the actual location of infection. In studies where the patient's residence was assumed to be the location of infection,⁷ there was only moderate concordance in time and space with surveillance indicators of transmission.⁸ Furthermore, because both competent avian and mosquito species are required for enzootic amplification, it has been unclear how their movements define the spatial dimensions of WNV transmission, especially after the primary avian hosts have fledged and birds are no lon-

ger faithful to breeding territories. The primary mosquito vector species in urban Los Angeles are *Cx. p. quinquefasciatus*, which has a flight range of ~1 km, and *Culex tarsalis* Coquillett and *Culex stigmatosoma* Dyar, which have flight ranges greater than 1 km in rural settings.^{9,10}

Because human cases have been a problematic metric for delineating transmission in time and space, a surrogate is needed so that the location and timing of infection can be identified with confidence. Caged chicken sentinels may provide a way to monitor tangential transmission events, but their use as a surrogate for human infection is unknown in Los Angeles. Previous analyses have indicated that sentinel chicken seroconversions were delayed relative to mosquito or dead bird infection data and therefore were not useful as an early warning of WNV transmission to humans.^{11–16} Similar studies have been contradictory, indicating that sentinel chickens were useful for WNV and other flavivirus surveillance applications.^{17–23} These conflicting results have led to divergence in opinion over the use of sentinel chicken data for risk assessment.

The current research analyzed the ability of sentinel chickens to track WNV transmission within Los Angeles, an urban continuum with annual human infection. Sentinel chicken seroconversion occurs concurrent with human case counts over a 6-year period, indicating that seroconversions tracked tangential rather than antecedent amplification transmission events. We then evaluated the ability of other surveillance indicators (temperature, dead bird reports, WNV-infected dead birds, WNV-infected mosquitoes, and free-ranging avian serology) to predict tangential transmission events in time and space. By controlling for spatial and temporal scale, we evaluated which surveillance indicators best predicted amplification using the transmission of WNV to sentinel chickens as a metric of tangential transmission.

METHODS

Our study areas in greater Los Angeles, California, data collection methods and time and space associations have been described recently¹⁶ and are summarized briefly below.

Description of Sites. Six sites within or near parkland embedded within Los Angeles were selected to cover the gradient from cool maritime (Machado Lake) to hot and dry

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inland valley habitats (Santa Clarita) and because of urban zoning regulations, security and the abundance of peridomestic birds (Figure 1). All sites were near residential areas, school campuses, parkland, and golf courses. The Machado Lake site (33°47'05" N, 118°17'35" W) is located in the city of Wilmington, California near the Sepulveda Drain and Harbor Lake constructed wetlands and is 5 km from the Pacific Ocean. The Whittier Narrows site (33°56'10" N, 118°03'54" W) is located in the city of South El Monte, California at the convergence of the Rio Hondo and San Gabriel Rivers near the Whittier Narrows Nature Reserve with a large American crow roost. The Rowland Heights site (33°58'53" N, 117°53'37" W) is located in the unincorporated city of Rowland Heights, California in the San Gabriel Valley, near the unimproved portion of Jellick Creek. The Los Angeles Zoo site (34°08'10" N, 118°17'01" W) is located in Griffith Park near the Los Angeles River. The Sepulveda Basin site (34°22'55" N, 118°33'58" W) is located in the San Fernando Valley in the incorporated community of Encino in the city of Los Angeles and is near Lake Balboa. The Santa Clarita site (34°10'27" N, 118°29'51" W) is located in the city of Santa Clarita, California in the Santa Clarita Valley surrounded by the Sierra Pelona, Santa Susanna, and San Gabriel mountains.

Surveillance data. *Sentinel chickens.* Flocks of 10 white leghorn hens that were 16–18 weeks of age were deployed annually and then maintained at each of the six sites

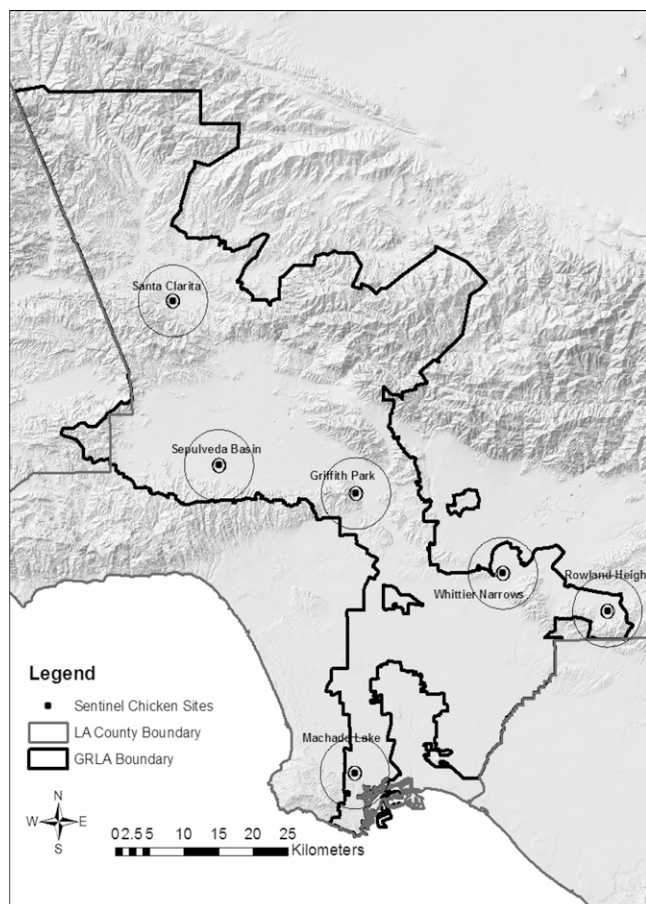


FIGURE 1. The sentinel chicken flock sites located within the Los Angeles County border with 1- and 5-km sampling zones used to aggregate the mosquito and dead bird sampling data.

described previously. Blood samples (0.1 mL) were collected every 2 weeks by brachial venipuncture and placed on filter paper strips.²⁴ The strips were sent to the California Department of Public Health, Viral and Rickettsial Disease Laboratory in Richmond, California for testing by EIA and immunofluorescence assay (IFA) for presence of antibody to WNV, western equine encephalitis virus (WEEV), and St. Louis encephalitis virus (SLEV).²⁵ Sentinel chickens develop WNV antibody ~7–10 days after experimental infection,^{26,27} but were bled on a biweekly (i.e., 2-week) cycle, which did not permit the precise determination of the time of WNV infection and seroconversion, requiring the evaluation of temporal lags of 1–14 and 15–28 days. Chicken flocks were replaced when five or more chickens seroconverted to WNV.

Human case reports. Human cases of West Nile fever and West Nile neuroinvasive disease were monitored by the Los Angeles County Department of Health and Human Services, Acute Communicable Disease Control, through passive case detection and reporting. Cases were limited to those that matched the Center for Disease Control and Prevention (CDC) definition for WNV-associated neuroinvasive or febrile illness and had been laboratory-confirmed, typically by demonstration of immunoglobulin M (IgM) antibody in sera or spinal fluid by EIA.²⁸ Additional human infections were discovered through blood donor programs and were included if they developed clinical symptoms.

Mosquito infection. Mosquitoes were collected biweekly near each sentinel chicken site, using dry ice-baited and gravid female traps, with additional collections occurring on a monthly trapping cycle, as described previously.¹⁶ Lots (pools) of < 50 females each were tested for WNV RNA using a real-time reverse transcription-polymerase chain reaction (RT-PCR) multiplex system.¹⁶ For the current analyses, pools were limited to those collected within 1- or 5-km sampling zones or buffers around each sentinel chicken site.

Dead birds. Dead birds found by the public were reported to the Department of Public Health, Vector-Borne Disease Section, Dead Bird Hotline.²⁹ If birds were considered to be in a testable condition, they were forwarded to the California Animal Health and Food Safety (CAHFS) laboratory at UC Davis for necropsy. Oral swabs (American Crows only) or kidney tissues were tested for WNV by singleplex RT-PCR. Dead bird monitoring and testing was conducted year-round.

Serology of free-ranging avian species. Free-ranging birds were collected by grain-baited Australian crow traps with inlet apertures set to be restrictive for small birds.³⁰ Traps were placed within 5 km of each of the six sentinel chicken sites and were set to collect birds for 24 hours bi-weekly.¹⁶ Sera were screened by EIA, with positives confirmed by a plaque reduction neutralization test as described previously.¹⁶ The seroconversion rate was defined as the number of new infections/total susceptible birds bled per bleed date. New infections here were identified as antibody-positive birds known from recapture data to have been negative at the previous bleeding. No time period was specified between blood sampling for this determination of seroconversion to an antibody-positive state. Cumulative seroprevalence was defined as the number of positive birds/total number of birds collected and tested on each bleed date.

Climate. Daily maximum and minimum temperatures were obtained from the National Aeronautics and Space Administration (NASA) Terrestrial Observation and Prediction

System (TOPS) that combines ground-based, remotely sensed, and modeled data inputs to produce climate surfaces for California. Daily data were aggregated into the 14-day intervals between sentinel chicken bleed dates. Degree-day accumulation was calculated using the single-triangle function estimation method,³¹ with a 14.3°C minimum threshold for WNV replication within the mosquito vector.³² A total of 109 degree-days were required for the completion of the extrinsic incubation period (EIP) from infection to transmission by *Culex* females.

Analyses. Temporal concordance between sentinel chicken seroconversions and human cases reported within the Greater Los Angeles Vector Control District jurisdiction was evaluated by time series graphs and cross-correlation analysis.

Sentinel chicken seroconversions were used as an outcome measure of tangential transmission to compare the antecedent predictive value of other surveillance indicators measured at the six study sites. Mosquitoes and wild birds were trapped on a 2-week cycle concurrent with chicken serosampling. The location of the sentinel chicken sites (Figure 1) and 1- and 5-km radius sampling zones or buffers were used to delineate surveillance data for analyses. The 1-km sampling zone reflected mosquito-driven transmission concordant with risk assessment models currently used in California^{7,9} that account for the average flight distance of the primary urban vector, *Cx. p. quinquefasciatus*.¹⁶ The 5-km sampling zone was used to exceed the 4.3 km maximum flight distance of the ornithophilic and highly competent vector, *Cx. stigmatosoma*,^{5,9,33–35} and to determine if avian factors increase WNV transmission beyond the area expected from mosquitoes alone. Larger buffers would result in overlap.

To determine whether multiple surveillance indicators of WNV transmission are associated with increased risk (or hazard) of seroconversion over time, the Cox regression analysis was used to model infection-free time. Analysis was based on days until sentinel chicken seroconversion or censoring. Censored times were defined as the time until removal caused by death (not related to WNV infection) or the end of the transmission season. Time for replacement chickens was counted from the first day that they were placed at the study site until they experienced seroconversion or removal. Predictor variables included infected mosquitoes, accumulated degree-days over 14.3°C, average minimum temperature, average maximum temperature, number of dead bird reports, number of WNV-positive dead birds, wild bird cumulative seroprevalence, and wild bird seroconversion rate. All

predictor variables were calculated for 1- and 5-km spatial sampling zones (buffers) at time lags of 1–14 and 15–28 days before seroconversion or removal caused by death or illness. Spatial relationships were set by drawing the 1- and 5-km sampling zones around each site in ArcMap 9.2 (ESRI, 2006), and appending the chicken site code to all datasets with values that fell within each of the sampling zones. All statistical analyses were performed with SAS version 9.1 (SAS Institute Inc., Chicago, IL, 2008). Individual group-specific “survival” curves (probability of survival or not seroconverting) were estimated using the Kaplan–Meier (KM) method. The KM curves were obtained for the presence/absence of positive mosquito pool detection (in 1- and 5-km spatial sampling zones), and by site and year. Differences between survival curves were compared using the log-rank test. Cox regression models were used to estimate adjusted relative risks of seroconversion for the four combinations of sampling zones and specific time periods of interest, 1 km and 1–14 days, 1 km and 15–28 days, 5 km and 1–14 days, and 5 km and 15–28 days before seroconversion detection. The Akaike information criterion (AIC) values and weights were compared among models to determine which had the best fit.³⁶

RESULTS

Surveillance data. Sentinel chickens. Overall, 143 sentinel chickens seroconverted to WNV, with 45 and 39 seroconversions occurring during the outbreak years of 2004 and 2008, respectively (Figure 2). Seroconversion prevalence varied from 11% to 44% among flock locations (Table 1). Observed times for chicken removal caused by death or illnesses before seroconverting to WNV were “censored times” in survival analysis. The proportions of chickens removed from the study were defined as “censored while in study” and were presented in Table 1. Those chickens that lived through the year and did not experience seroconversion were “censored at end of study,” because they were replaced annually with seronegative chickens each spring. Except for 2005, at least one sentinel chicken flock was replaced each year because of the seroconversion of > 50% of the chickens within the flock.

Human case reports. A time-series graph of human cases plotted at onset date and sentinel chicken seroconversions plotted by detection date revealed temporal concordance (Figure 2), although the amplitude for chicken seroconversions was lower than human case counts probably because of the limited number of chickens monitored. The strength of this

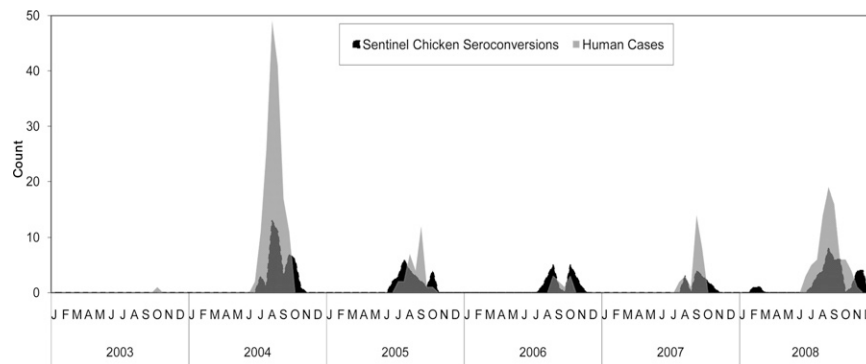


FIGURE 2. Biweekly sentinel chicken seroconversions and human cases for the study years 2003–2008.

TABLE 1

Proportions of chickens by study site and year that were censored in one of three ways: 1) removal while in the study (typically caused by illness or death before experiencing seroconversion), 2) replacement after seroconversion, and 3) end of the surveillance year without seroconversion*

Site	Removal from study	Seroconversion	End of study
Sepulveda Basin	0.06	0.35	0.59
Griffith Park	0.01	0.37	0.61
Machado Lake	0.20	0.11	0.70
Rowland Heights	0.07	0.44	0.49
Santa Clarita	0.15	0.37	0.48
Whittier Narrows	0.13	0.11	0.77
Year			
2004	0.15	0.27	0.58
2005	0.07	0.30	0.63
2006	0.01	0.31	0.68
2007	0.09	0.21	0.70
2008	0.09	0.21	0.70

*Values may not sum to 1 because of rounding.

concordance was evaluated by cross-correlation analysis by holding human case counts constant by date of onset and shifting the sentinel chicken conversions progressively earlier or later in time. Because of the seasonal trend present in both data sets, the time period was restricted to the months of June–November, when there were nonzero values in one of the datasets. The cross-correlation coefficients with 95% confidence intervals (CIs) (Figure 3) revealed that sentinel chicken seroconversions were marginally associated with human cases 4 weeks before their occurrence ($r = 0.22$, $P = 0.056$), significantly associated 2 weeks before case occurrence ($r = 0.47$, $P < 0.001$), highly correlated during the same time period ($r = 0.74$, $P < 0.001$), and remained significantly correlated up to 6 weeks after human case occurrence ($r > 0.34$, $P < 0.005$). Elevated temporal concordance indicated that both measures concurrently tracked the same tangential or “spill-over” transmission events.

Mosquito infection. There were 53 mosquito trap sites within the 1-km sampling zones and 171 sites within the 5-km sampling zones around sentinel chicken flocks. The numbers of trap nights, mosquito pools tested, and WNV-positive pools were combined over *Culex* species and summarized in Table 2. Because of the low numbers of positive pools within each time

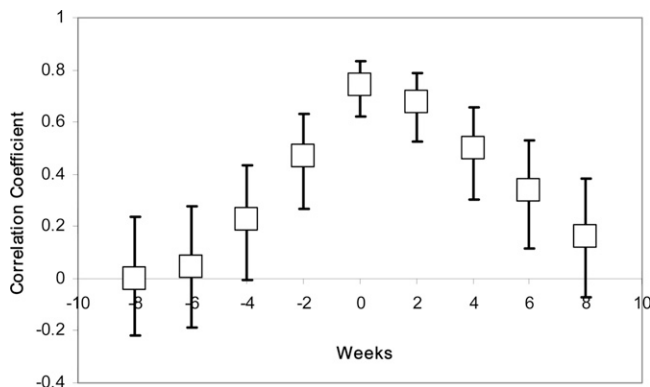


FIGURE 3. Correlation coefficients with 95% confidence intervals between human case counts and lagged sentinel chicken seroconversions (lag is represented on x axis). Human case counts were held constant by date of onset, whereas the sentinel chicken seroconversions were lagged backwards or forwards by weeks as indicated on the x axis.

TABLE 2

Mosquito sampling effort and West Nile virus (WNV) infection (all *Culex* combined) within the 1- and 5-km spatial sampling zones around sentinel chicken cages by year

Year	1 km			5 km		
	Trap nights	Pools submitted	Positive pools (%)	Trap nights	Pools submitted	Positive pools (%)
2004	786	630	47 (7)	2030	1137	121 (11)
2005	713	426	11 (2)	1871	1057	27 (3)
2006	688	220	7 (3)	1950	715	60 (8)
2007	567	407	5 (1)	1911	1792	45 (3)
2008	461	302	22 (7)	1521	950	142 (15)
Totals	3215	1985	92 (5)	9283	5651	395 (7)

period, this variable was converted to a dichotomous positive or negative outcome for KM analyses.

Dead birds. Overall, American Crows and House Finches comprised 60% and 6% of the 4,205 dead birds tested, respectively.¹⁶ The numbers of reported, tested, and WNV RNA-positive dead birds were summarized by year in Table 3 for 1- and 5-km spatial sampling zones around each of the flocks. The numbers of birds reported, tested, and positive were greatest during the epidemic of 2004 and then progressively declined over the study period. When stratified by location (Table 4), two sites near crow roosts (Sepulveda Basin and Whittier Narrows) had over two-thirds of the dead birds tested.

Serology of free-ranging avian species. The seroconversion rates and cumulative seroprevalence of House Finches and House Sparrows (97% of 14,107 sera tested) were similar temporally¹⁶ and were combined for analyses and in Figure 4. The maximum cumulative seroprevalence observed was 0.46 in November 2004, following the first WNV epidemic in Los Angeles. As anticipated, cumulative seroprevalence generally increased over the transmission season and was greatest during the subsequent winter months. Recruitment of hatching year birds decreased seroprevalence during summers with limited WNV activity. Trends in the seroconversion rate were less intuitive, because high rates were observed outside of the transmission season, most likely caused by increased trap affinity by birds foraging during the winter months. Detailed analysis of the avian serology will be presented separately.

Climate. Mean minimum temperatures ranged between 0.17 and 26.1°C and mean maximum temperatures between 7.9 and 37.7°C. Whittier Narrows was the first site to reach the estimated 109 degree-day threshold necessary for WNV extrinsic incubation³² in all the study years, followed by Griffith Park, Sepulveda Basin, and Rowland Heights.

TABLE 3

Dead bird reports and West Nile virus (WNV) test results per year within the 1- and 5-km spatial sampling zones around sentinel chicken flocks

Year	1 km			5 km		
	Reports*	Tested	Positive (%)	Reports*	Tested	Positive (%)
2004	71	24	18 (75)	3693	371	224 (60)
2005	19	6	4 (67)	472	97	45 (46)
2006	17	9	3 (33)	253	78	16 (20)
2007	8	4	1 (25)	234	97	26 (27)
2008	6	2	2 (100)	132	80	33 (41)
Totals	121	45	28 (62)	4784	723	344 (48)

*Dead birds reported by the public were forwarded for testing if they were reported within 24 hours of death and the carcass was intact.

TABLE 4
Dead bird reports by site and West Nile virus (WNV) test results

Site	Reports*	Tested	Positive (%)
Sepulveda Basin	2,401	269	161 (60)
Griffith Park	482	95	20 (21)
Machado Lake	393	46	16 (35)
Rowland Heights	432	53	20 (38)
Santa Clarita	410	142	48 (34)
Whittier Narrows	1,046	174	100 (57)

*Dead birds reported by the public were forwarded for testing if they were reported within 24 hours of death and the carcass was intact.

Degree-day accumulation in Santa Clarita did not achieve the EIP threshold in 2004, but did in all other study years. The Machado Lake site did not achieve the EIP threshold in 2005 or 2008; 2007 was the only year that it remained above the minimum threshold for greater than a 2-week period.

Analyses. Survival analysis. Sentinel chicken seroconversions were not detected during the 2003 surveillance year when WNV invaded the Los Angeles Basin,¹⁶ so analyses and Cox regression models were restricted to the 2004–2008 period. Kaplan–Meier survival curves stratified by site are presented in Figure 5A. Flocks at Sepulveda Basin, Griffith Park, and Rowland Heights had significantly ($P < 0.001$) lower overall median infection-free times when compared with those at other sites, indicating that transmission depended on location. Therefore, site was included in subsequent Cox regression models. Comparisons of infection-free time were not statistically different between Sepulveda Basin and Griffith Park sites ($P = 0.98$) or between Sepulveda Basin and Rowland Heights ($P = 0.09$). The infection-free times at Machado Lake, Santa Clarita, and Whittier Narrows were similar ($P = 1.0, 0.993, 1.0$, respectively). The Kaplan–Meier survival curves for each year are presented in Figure 5B (with the censored proportions presented in Table 1). Comparisons between years revealed that epidemic years 2004 and 2008 ($P = 0.997$) were not statistically different from each other, but were statistically different from years 2005, 2006, and 2007 with low WNV transmission that were statistically similar ($P = 1.0, 0.975, 0.952$).

After mosquito pool samples were limited to the spatial sampling zones and specific antecedent time periods, sample sizes were too small to calculate infection incidence, therefore the mosquito infection variable was converted to a dichotomous variable for the presence or absence of a WNV-positive mosquito pool. Kaplan–Meier survival curves for presence/absence of positive mosquito infection within 1- or 5-km sampling zones around each site are presented in Figures 5C and D,

respectively. The infection-free time with WNV-positive mosquito detection was significantly different from time periods without mosquito infection for both spatial scales ($P < 0.001$ and $P = 0.027$).

Cox regression models. Table 5 summarized four multiple Cox regression models of infection-free time in chickens, constructed on the basis of data for the two spatial and temporal lags. Relative risks (RRs) less than 1.0 were suggestive of protective effects, as in the case of surveillance indicators that occurred in the low WNV activity year 2007 ($RR < 0.135$) and the Wild Bird Seroconversion Rate ($RR < 0.408$). The dichotomous variables with higher RRs were associated with increased risk of seroconversion, whereas RRs for continuous variables were compared with a baseline value. Of interest were transmission indicators that shifted between being significantly dependent on the lag intervals. For example, the predictor Wild Bird Seroconversion Rate appeared in all four models, but shifted between significance and marginal significance (1 km and 1–14 day, $P = 0.103$; 1 km 15–28 day, $P = 0.006$; 5 km 1–14 day, $P = 0.015$; 5 km 15–28 days, $P = 0.004$). Wild Bird Cumulative Sero-prevalence was not significant ($P = 0.116$) in the 5 km 1–14 day model, but was suggestive of an association, as were Dead Bird Reports ($P = 0.074$) in the 1 km 1–14 day model. In contrast, Positive Dead Birds was significant only in the 5 km 15–28 day model. The variable Positive Mosquito Pool was marginally significant ($P = 0.075$) only in the 5 km and 15–28 day model. These findings indicated that individual enzootic surveillance variables have unique patterns in their ability to predict sentinel chicken seroconversions in space and time.

The AIC measures of model fit were 462.9 for the 1 km and 1–14 day time lag, 511.7 for the 1 km and 15–28 day time lag, 463.5 for the 5 km and 1–14 day time lag, and 456.1 for the 5 km and 15–28 day time lag. Differences in AIC and AIC weights for the models were compared, and the 5 km 15–28 day lag model was considered uniformly best with an AIC weight of 0.94. Relative risks for increases in each continuous variable in the most distal (5 km and 15–28 days), and best fitting model as measured by AIC, are presented in Table 6 as a measure of risk. Heat accumulation to 109 degree-days above 14.3°C had the greatest impact on the infection-free time of sentinel chickens and raised the RR of seroconversion to 7.99 times the RR of the minimum observed value, or baseline, of 0 degree-days. Mean maximum temperature had the second greatest impact on increasing RR, with a 3°C increase resulting in a 3.05 times greater risk. The variable Positive Dead Bird was zero-enriched; leading both the median and third quartile

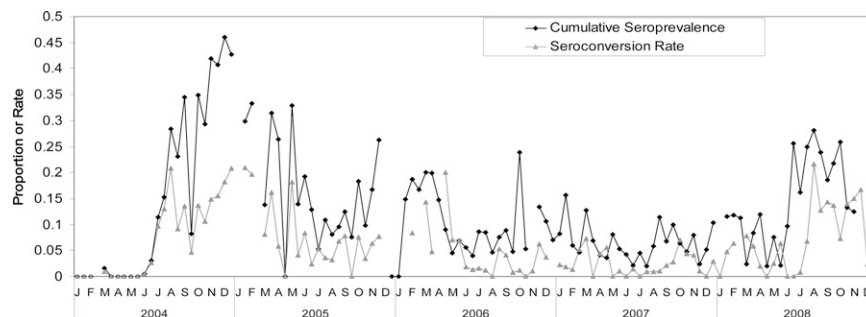


FIGURE 4. Cumulative seroprevalence (number positive/total collected) and seroconversion rate (number newly positive/total susceptible birds collected during each 2-week interval) for house finches and house sparrows collected in Los Angeles County during 2004–2008.

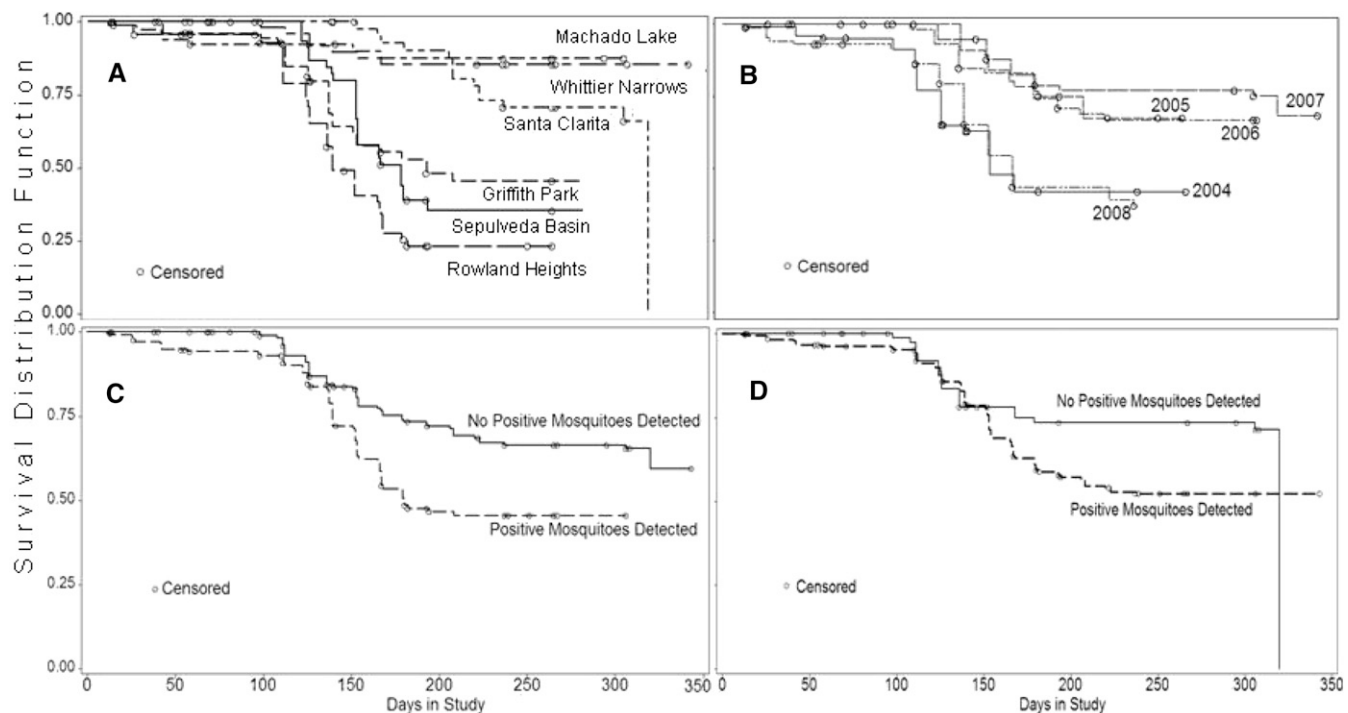


FIGURE 5. Kaplan-Meier curves for time to sentinel chicken seroconversion stratified by (A) site, (B) year, (C) the presence of a positive mosquito pool within 1 km of the sentinel chicken site (log rank test, $P = 0.001$), and (D) the presence of a mosquito pool within 5 km of the sentinel chicken site (log rank test, $P = 0.0272$).

to equal 0. An increase above two positive dead birds in an interval increased risk 1.15 times the baseline (Table 6).

DISCUSSION

Sentinel chicken seroconversions tracked WNV tangential transmission during outbreaks in Los Angeles, because infection-free time of sentinel chickens was statistically similar during the 2004 and 2008 epidemics.¹⁶ Furthermore, sentinel chicken seroconversions and human cases were temporally concordant, even with sampling intervals and intrinsic incubation periods confounding seroconversion dates and the onset of disease, respectively. Detection precision of seroconversion dates could be improved by shortening the intervals between blood sampling from 2 weeks to 1 week. Our current and previous analyses indicated that although sentinel chickens were not necessarily a valuable early season enzootic predictor of human cases,¹⁶ they provided a highly significant indication of concurrent WNV tangential transmission to humans. Concordance of human cases and sentinel chicken seroconversions has been noted in other areas with WNV activity,¹⁵ including areas where *Cx. p. quinquefasciatus* was the primary tangential vector,^{11,37} although in the latter paper, concordance was only observed in the third year of WNV activity.

Although WNV amplification in urban environments is typically very rapid,^{13,16} the passive surveillance system monitoring human WN disease often has delays in reporting of human cases to public health and mosquito control agencies. These problems were compounded in Los Angeles where an ethnically diverse human population of > 10 m sought care from a mosaic of medical providers and diagnostic laboratories, creating geographic, jurisdictional, and linguistic challenges for

epidemiologic surveillance. Therefore, knowledge of the risk for tangential virus transmission using a sentinel chicken surrogate for human cases could be very useful for implementing emergency control and public education.

Accurate prediction of tangential transmission events may extend response time and thereby improve the ability of mosquito control to interrupt transmission and preclude human disease. The most significant predictors of sentinel chicken seroconversions were surveillance parameters measured within a 5-km-radius sampling zone centered at the sentinel chicken flock site, with an antecedent time lag of 15–28 days. The spatial buffer of 5 km included more surveillance data, such as mosquito trap sites and dead bird reports from the surrounding area than was encompassed by the smaller 1-km buffer. The larger zone reflected a greater flight range of *Cx. tarsalis* and *Cx. stigmatosoma* that are more competent vectors and more dispersive^{5,9} than *Cx. p. quinquefasciatus*, but are less abundant in urban Los Angeles and collected less frequently by our trapping methods.¹⁶ The antecedent time period of 15–28 days before seroconversion may more accurately capture epizootic amplification leading to tangential transmission. Similar findings may be true in even larger spatial and temporal windows; however, at the scale of our current study larger spatial zones would result in overlap between adjacent zones around the sentinel sites.

Positive mosquito pools were associated only marginally with sentinel chicken seroconversions; however, this most likely was caused by the small sample sizes mentioned previously. The dead bird reports and positive test results were interesting in that positive test results were most predictive in the most distal (5 km, 15–28 days prior) model, whereas reports were predictive only in the most proximal (1 km, 1–14 days

TABLE 5

Relative risk of seroconversion estimated from four Cox regression models*

Variable	P value	Relative risk
1-km model 1–14 days prior		
Year 2004	< 0.001	5.860
Year 2007	< 0.001	0.135
Year 2008	0.003	3.161
Wild bird seroconversion rate	0.103	0.408
Dead bird reports	0.074	1.130
Average minimum temperature	0.046	1.318
Degree days	0.018	1.025
1-km model 15–28 days prior		
Year 2005	0.057	0.474
Year 2006	< 0.0001	0.117
Year 2007	< 0.0001	0.12
Wild bird seroconversion rate	0.0061	0.032
Average maximum temperature	0.0258	1.283
Degree days	0.0429	1.019
5 km model 1–14 days prior		
Year 2004	< 0.0001	5.893
Year 2007	0.0004	0.126
Year 2008	0.0038	3.062
Wild bird cumulative seroprevalence	0.1162	3.147
Wild bird seroconversion rate	0.0145	0.166
Average minimum temperature	0.0532	1.308
Degree days	0.0113	1.027
5 km model 15–28 days prior		
Year 2004	< 0.001	9.896
Year 2005	0.016	3.656
Year 2008	< 0.001	14.028
Wild bird seroconversion rate	0.004	0.019
Positive mosquito pool	0.075	0.452
Positive dead bird	0.002	1.074
Average maximum temperature	0.004	1.451
Degree days	0.049	1.019

*The most proximal model to sentinel chicken seroconversions included covariates within the 1-km spatial sampling radius and the 1–14 day antecedent temporal lag, whereas the most distal model included covariates from within the 5-km spatial sampling zone and the 15–28 day antecedent temporal lag.

prior) model. These latter differences may reflect enhanced statistical power, because more dead birds were reported than tested for WNV.

Wild bird seroconversions (a temporally imprecise surrogate for infection incidence) consistently provided a “protective effect” on sentinel chicken seroconversions. This effect may have been caused by the timing of wild bird recapture, because not all wild birds were recaptured during every interval. Imprecision in the time of free-ranging bird seroconversion was evident by seroconversion rates above zero during winter months when there were no other indications of transmission. When this variable was compared with cumulative seroprevalence in wild birds in time series (Figure 4), it appeared that the seroconversion rate mirrored the temporal trend in cumulative seroprevalence that removed variance because of bird survivorship. When the seroconversion rate approached one the estimated RR was 0.02, an effect that indicated nearly complete protection of chickens from seroconversion. Such protection would result in the cessation of WNV transmission caused by herd immunity in the principal reservoir hosts.

Average maximum temperatures and accumulated degree-days were more effective at the long time lag (15–28 days) in both of the spatial sampling zones as indicated by significant correlation coefficients. In the short time lag (1–14 days), the average minimum daily temperatures and degree-days were most predictive of sentinel chicken seroconversions. This

TABLE 6

Relative risk associated with increases in continuous variables for the best fitting Cox regression models, using the 5-km sampling zone and data collected 15–28 days before the sentinel chicken seroconversions

Variable	Increase	Regression coefficient	Relative risk	SE	95% CI	
					Lower	Upper
Wild bird seroconversion rate		−3.949	0.019	1.36		
	0.00		1.00		0.07	14.48
	0.10		0.67		0.05	9.76
	0.25		0.37		0.03	5.40
Positive dead bird		0.072	1.07	0.023		
	0		1.00		0.96	1.05
	1		1.07		1.03	1.12
	2		1.15		1.10	1.21
Mean maximum temperature		0.372	1.45	0.128		
	1		1.45		1.13	1.86
	3		3.05		2.38	3.92
	5		6.43		5.00	8.26
Degree days		0.019	1.019	0.010		
	59		3.08		3.02	3.14
	109		7.99		7.84	8.15
	159		20.74		20.35	21.14

*Increase refers to a change in the continuous variable, whereas relative risk is the exponentiated risk for the given value and coefficient, 95% CI is the confidence interval for the relative risk estimate.

finding is similar to those for WEEV transmission by *Cx. tarsalis* to sentinel chickens in California, where maximum temperatures were predictive 1–3 weeks before seroconversion.³⁸ Increases in the minimum temperature increased the accumulation of suitable degree-days for viral amplification³⁹ which, in turn, accelerated local transmission. This point was emphasized by the sites at Griffith Park, Whittier Narrows, and Sepulveda Basin achieving the minimum EIP earliest and also experiencing the greatest WNV activity, whereas Machado Lake rarely achieved the minimum EIP and had the least WNV activity. Additional supporting evidence is the site-specific patterns in infection-free time, where sites with higher temperature and degree-day accumulations also had lower median infection-free times.

The resurgence in WNV activity in 2008 provided an opportunity to examine similarities between the two epidemic years and look for surveillance indicators that continued to predict transmission activity after WNV became endemic within this urban area. In the current study, sentinel chickens provided a highly correlated measure of tangential transmission and an excellent surrogate for human case information before epidemiologic reporting. By looking at enzootic predictors in time and space for this outcome measure, we found that larger spatial and earlier temporal scales provided a more accurate assessment and that endemic transmission was positively associated with temperature while mitigated by wild bird immunity. These results have been incorporated into decision support paradigms for escalating WNV intervention by the Greater Los Angeles County Mosquito and Vector Control District focusing on mosquito control and public education.

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